Abstract. *Myrmecodia platytyrea* Becc., a member of the Rubiaceae family, is found throughout Southeast Asia and has been traditionally used to treat cancer. However, there is limited pharmacological information on this plant. We investigated the anticancer effects of the methanol extract of *Myrmecodia platytyrea* Becc. leaves (MMPL) and determined the molecular mechanisms underlying the effects of MMPL on metastasis in human hepatocellular carcinoma (HCC) cells. MMPL dose-dependently inhibited cell migration and invasion in SK-Hep1 and Huh7 cells. In addition, MMPL strongly suppressed the enzymatic activity of matrix metalloproteinases (MMP-2 and MMP-9). Diminished telomerase activity by MMPL resulted in the suppression of both telomerase activity and telomerase-associated gene expression. The levels of urokinase-type plasminogen activator receptor (uPAR) expression as well as the phosphorylation levels of signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK) were also attenuated by MMPL. The above results collectively suggest that MMPL has anticancer effects in HCC and that MMPL can serve as an effective therapeutic agent for treating human liver cancer.

Introduction

Cancer is one of the greatest public health burdens worldwide, causing abnormal cell growth through DNA mutation that often leads to death (1). Metastatic tumors, in particular, result in 90% of human cancer deaths (2). Cancer metastasis contributes to both morbidity and mortality, and it is the most challenging target of effective cancer treatment (3). Metastatic cells detach from the primary tumor, travel to other sites through the circulatory or lymphatic system, and adhere and proliferate in distant organs, resulting in highly incurable and fatal diseases (3,4). Recent research has focused on understanding the precise mechanism of metastasis in order to prevent various steps in the process (5).

In addition, there is emerging evidence that telomerase is a promising target for the development of new cancer therapies (6,7). Elevated telomerase activity has been found in approximately 90% of cancer cells (8). Cancer cells can be immortalized by maintaining the telomere length, which is regulated by telomerase. Reactivation or increased expression of telomerase leads to continuous proliferation of cancer cells (9).

Natural products have been used in human medicine since ancient times. For several decades, plants and plant-derived compounds have been used to combat cancer often with fewer side effects than traditional treatments (10). Since plants have been a useful source of approved anticancer agents, many efforts have been undertaken to investigate the wide range of pharmacological effects of plants against various types of cancer (11,12).

*Myrmecodia* (Rubiaceae) is a genus of the epiphytic myrmecophytes or ant-plant, which is locally known as Sarang Semut. This genus is native to Southeast Asia, but it can also be found in Myanmar, Indochina, and northern Queensland in Australia (13). *Myrmecodia* plants contain a highly specialized tuber and modified stems, which are used by ant colonies (14). *Myrmecodia* species have traditionally been used to treat diseases such as ulcers, tumors, cancer, hepatitis, and coronary artery disease (15).

In pharmacological studies, *Myrmecodia pendens* and *tuberose* are both considered to be potential anticancer agents. Water extracts of *Myrmecodia pendens* have been shown to inhibit HeLa and MCM-B2 cancer cell growth (16). Five flavonoids of *Myrmecodia pendens*, kaempferol, luteolin, rutin, quercetin, and apigenin, were identified and quantified, and...
some or all of these phenolic compounds may have anticancer properties (17). Ethanol extracts of *Myrmecodia tuberose* have been shown to suppress tumor growth and induce apoptosis of oral carcinoma cells (18). In addition, this plant extract significantly increased macrophage phagocytosis and lymphocyte proliferation in doxorubicin-induced immuno-suppressed rats (19). Since one of the main side effects of doxorubicin in cancer chemotherapy is immunosuppression, this research supports the notion that *Myrmecodia tuberose* could be used as a complementary agent for cancer treatment by improving immune responses (20,21).

Although *Myrmecodia platytyrea* Becc. is commonly used for cancer treatment by local residents throughout Southeast Asia, there has been little scientific investigation about its pharmacological activity. Bioactive compounds such as stigmasterol, morindolide, and flavonoids have been found in the tuber of the plant (22). The tuber extract has demonstrated toxic effects on HepG2 cells without affecting normal monkey kidney cells, suggesting this extract inhibits the growth of human hepatocellular carcinoma (HCC) (23). However, very little is known about the biological activity of *Myrmecodia platytyrea* Becc. leaves. This study investigated the potential anticancer activity of the methanol extract of *Myrmecodia platytyrea* Becc. leaves (MMPL) in HCC cells and the mechanism underlying these effects.

**Materials and methods**

**MMPL preparation.** The methanol extract of *Myrmecodia platytyrea* Becc. leaves (Voucher no. KRIIBB0047070) was purchased from the International Biological Material Research Center in Korea Research Institute of Bioscience and Biotechnology (KRIBB, http://www.ibmrc.re.kr, Daejeon, Korea). A voucher specimen (KRIIBB0047070) was deposited at KRIIBB. The extract was diluted in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) and added directly to the culture media. To avoid cell damage, the final concentration of DMSO never exceeded 1%.

**Cell culture and reagents.** Human hepatoma cell lines with different metastatic potentials, including SK-Hep1 with high metastatic potential and Huh7 with low metastatic potential (Korean Cell Line Bank, Seoul, Korea), were maintained in Dulbecco’s modified Eagle’s medium (DMEM) or Roswell Park Memorial Institute 1640 (RPMI-1640) supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin and 50 µg/ml streptomycin (Gibco-BRL, Grand Island, NY, USA) at 37°C in a humidified 5% CO₂ atmosphere.

**Cell viability assay.** Cells were seeded in 24-well plates (1.5x10⁴ cells/well) and treated with various concentrations of MMPL for either 24 h or 48 h with media containing 1% or 10% FBS. Cell viability was evaluated using EZ-Cytosol solution (Daeil Lab, Seoul, Korea) according to the manufacturer’s instructions. Briefly, EZ-Cytosol solution was added to each well and the plate was incubated for 1 h at 37°C. The optical density (OD) of the supernatants was measured at 450 nm using a Synergy H1 Microplate Reader (BioTek Instruments, Winooski, VT, USA).

**Wound healing assay.** SK-Hep1 and Huh7 cells were seeded in 6-well plates (0.5x10⁶ cells/well) and cultured until they reached 90% confluence. The wound area was created using a scratcher tip (0.5 mm; SPL Life Sciences, Gyeonggi-do, Korea). The detached cells were removed with phosphate-buffered saline (PBS) supplemented with media containing 1% FBS and treated with the indicated concentration of MMPL for 24 h. The cells that migrated into the wound surface were observed using a JuLI Stage Real-Time Cell History Recorder (NanoEnTek Inc., Seoul, Korea). The change in wound closure is represented as the percent of wound recovery. All experiments were performed in triplicate.

**Transwell invasion and migration assay.** SK-Hep1 cells (5x10⁵ cells/well) in 250 µl of media containing 1% FBS were added to the upper chamber with or without MMPL, and the lower chamber was filled with 500 µl of media containing 10% FBS. The plates were incubated at 37°C and 5% CO₂ for 18 h. For the Matrigel invasion assay, 24-Transwell plates (pore size, 8 µm) were coated with 30 µl of diluted Matrigel (0.5 mg/ml; BD Biosciences, Franklin Lakes, NJ, USA) for 2 h, and the cells were seeded onto the insert with or without MMPL for 18 h. The membranes to which cells migrated were fixed in 3.7% paraformaldehyde in PBS, and cells adhering to the upper surface of the membrane were removed with a cotton swab. The invasive cells were stained with 0.5% crystal violet. After being dried, five random fields of the membrane were observed and counted by averaging the total number of cells at x100 magnification.

**Luciferase assay.** A luciferase reporter plasmid containing the uPAR (−682/+27) promoter region (pcDNA3.1-uPAR (−682/+27)-luc) was constructed with the insertion of a 709 bp polymerase chain reaction (PCR) product (using the specific primers: sense, 5’-GGG GCT AGA TCT GAT TCT CCT GCC TCA GCC TC-3’ with BglII site and antisense, 5’-CGG GAA TTC GGG TCC CTG CAC GTC TTC TC-5’ with EcoRI site) into the BglII and EcoRI sites of the plasmid pcDNA3.1-luc, which contains the luciferase gene. For luciferase assays, the cells were co-transfected with pcDNA3.1-uPAR (−682/+27)-luc and the mechanism underlying these effects.
along with a gWIZ-green fluorescence protein (GFP) plasmid using polyethyleneimine (PEL, Polysciences, Inc., Warrington, PA, USA); transfected cells were then divided into 12-well plates (1x10^4 cells/well). After 24 h of incubation, cells were treated with various concentrations of MMPL for 24 h. Cells were then lysed with passive lysis buffer (Promega, Madison, WI, USA) and promoter activity was assayed according to the manufacturer's instructions. Cell lysates were assayed for GFP expression and the relative luciferase activity was normalized against the levels of GFP expression.

Gelatin zymography. The activity of matrix metalloproteinases (MMP-2 and MMP-9) was assayed by gelatin zymography (24). Briefly, SK-Hep1 cells (3.5x10^6 cells/well) were seeded in 6-well culture plates and incubated in serum-free medium in the presence of indicated concentrations of MMPL for 24 h, and the cultured media were collected. The samples were mixed with 5X sample buffer [312.5 mM Tris-Cl (pH 7.5), 0.2 M NaCl, 5 mM CaCl_2, 0.1 mM ZnCl_2, and 2.5% Triton X-100] twice for 30 min at room temperature (RT) and then incubated in substrate buffer [50 mM Tris-Cl (pH 7.5), 0.2 M NaCl, 5 mM CaCl_2, 0.1 mM ZnCl_2, 0.016% NaN_3, and 1% Triton X-100] at 37°C for 18 h. The gels were fixed with fixing solution (40% methanol and 10% acetic acid) for 30 min and then stained with staining solution (0.25% Coomassie brilliant blue R-250, 45% methanol, and 10% acetic acid) for 1 h, followed by destaining with destaining solution (5% methanol and 8% acetic acid). The zymography gels were scanned using an HP Photosmart image scanner (HP Development Co., L.P., Palo Alto, CA, USA). The band intensities were quantified using LabWorks image scanner (HP Development Co., L.P., Palo Alto, CA, USA) and promoter activity was assayed according to the manufacturer's instructions. Cell lysates were assayed for GFP expression and the relative luciferase activity was normalized against the levels of GFP expression.

**Statistical analysis.** All data represent three independent experiments and are expressed as means ± standard error of the mean (SEM). Statistical analyses were performed with a one-way ANOVA of Dunnett's Multiple Comparison method using Prism 3.0 (GraphPad Software, San Diego, CA, USA) and p<0.05, p<0.01, and p<0.001 were considered to indicate statistically significant.
Results

**Effects of MMPL on cell viability.** To investigate the cytotoxic effect of MMPL on hepatoma cells, SK-Hep1 and Huh7 cells were incubated with various concentrations of MMPL (0, 20, 40, 60 and 100 µg/ml) for 24 h in cell culture media with 1% or 10% FBS. Cell viability was examined using EZ-Cytox solution and the effect of MMPL on cell viability is expressed as a percentage of the control (Fig. 1). For SK-Hep1 cells, the results showed little or no effect on cell viability in cell culture media with 1% and 10% FBS and ≤80 µg/ml of MMPL (Fig. 1A). For Huh7 cells, MMPL slightly inhibited cell viability in a dose-dependent manner but it did not induce cytotoxicity until the concentration was >40 µg/ml (Fig. 1B). Therefore, MMPL concentrations ≤80 µg/ml in SK-Hep1 cells and 40 µg/ml in Huh7 cells were used throughout this study to eliminate the effect of MMPL-induced cytotoxicity on cell migration and invasion.

**Inhibitory effects of MMPL on cell migration and invasion.** Tumor progression requires cells to migrate and induce degradation of the extracellular matrix (ECM) or basement membrane (BM) for efficient cell invasion (30). Wound healing assays were performed to evaluate the effect of MMPL on cell migration. Cells were treated with various concentrations of MMPL and the cell confluence within the wound regions was measured using a Real-Time Cell History Recorder. The results showed that MMPL significantly suppressed wound closure in a dose- and time-dependent manner (Fig. 2A and B). When SK-Hep1 cells were treated with MMPL for 24 h, the percentage of wound recovery was decreased from 57.2±4.6% (0 µg/ml) to 30.9±1.8% (60 µg/ml) (Fig. 2A). For Huh7 cells, MMPL also inhibited wound recovery in a dose-dependent manner from 40.3±2.3% (0 µg/ml) to 15.4±1.9% (40 µg/ml) (Fig. 2B).

To further investigate the effect of MMPL on the motility of SK-Hep1 cells, migration and invasion assays were performed using a Transwell chamber. As shown in Fig. 2C, the result indicated that MMPL inhibited the migration of SK-Hep1 cells in a dose-dependent manner compared to the control group; the motility decreased by 54.8±9.5% after treatment with 60 µg/ml MMPL.

**Inhibitory effects of MMPL on MMP-2 and MMP-9 activity.** In cancer progression, MMPs are essential for the facilitation of cellular metastasis (32). Through the urokinase-type plasminogen activator (uPA)-uPAR signaling pathway, the expression and activation of MMPs are promoted, which enhances ECM degradation (33). Among MMPs, MMP-2 and MMP-9 are highly activated type IV collagenases which play critical roles in tumor invasion and metastasis by facilitating enzymatic digestion of the BM (34-36). Thus, the enzymatic activity of MMP-2 and MMP-9 was measured using gelatin zymography after SK-Hep1 cells were treated with various concentrations of MMPL (0, 20, 40 and 60 µg/ml) for 24 h. As shown in Fig. 3A, the constitutive secretion of low levels of MMP-2 and high levels of MMP-9 by untreated SK-Hep1 cells was observed. When cells were treated with MMPL, the activity of MMP-2 and MMP-9 was significantly inhibited in a dose-dependent manner. These results indicate that the inhibitory effect of MMPL on cell migration and invasion might be due to the downregulation of MMP-2 and MMP-9 activity.

**Inhibitory effects of MMPL on telomerase activity.** Recently, telomerase has been considered critical for carcinogenesis (9,37). To investigate whether MMPL inhibits telomerase activity, SK-Hep1 cells were treated with MMPL. Subsequently, the TRAP assay was performed using cell lysates to determine telomerase activity. As shown in Fig. 3B, telomerase activity was suppressed by MMPL in a dose-dependent manner. 5-FU, which was used as a positive control (38), also inhibited telomerase activity.

The BM and ECM environments, composed of collagen, fibronectin, and laminin, are the first barriers that cancer cells must overcome for invasion (31). Thus, invasion assays were conducted using a Matrigel-coated Transwell chamber to determine whether MMPL reduces the invasive potency of SK-Hep1 cells. MMPL significantly inhibited the invasion of SK-Hep1 cells in a dose-dependent manner (Fig. 2C). Results showed that cell invasion decreased by 54.7±5.1% after treatment with 60 µg/ml MMPL. Taken together, these results suggest that MMPL has an inhibitory effect on the metastatic properties of hepatoma cell lines.
Figure 2. Effects of MMPL on cell migration and invasion. After SK-Hep1 and Huh7 cells were scratched with a scratcher tip, (A) SK-Hep1 cells with MMPL (0, 20, 40 and 60 µg/ml) and (B) Huh7 cells with MMPL (0, 10, 20 and 40 µg/ml) were incubated in cell culture media containing 1% FBS for 24 h. Cell motility was quantified by measuring the percent of wound recovery area compared to the zero time-point. (C) SK-Hep1 cells with MMPL (0, 20, 40 and 60 µg/ml) in media containing 1% FBS were seeded into upper chamber wells that were coated with or without the Matrigel. The lower chambers were filled with media containing 10% FBS. After incubation for 18 h, cells that penetrated the lower surface of the filter were fixed and stained with crystal violet. Quantification of migrated and invaded cells was performed by counting cells in five random fields. Data shown are a representative of three experiments and expressed as the means ± SEM (n=3). *p<0.05 and **p<0.001 relative to the MMPL-untreated control group.
To determine whether MMPL can directly suppress the enzymatic activity of telomerase, a telomerase activity assay was carried out after cell lysates were treated with MMPL. The results presented in Fig. 3C show a significant decrease in telomerase activity by MMPL compared with the known telomerase inhibitor BIBR1532 (39).

Telomerase has two core components, hTERT and hTR (40). When SK-Hep1 cells were treated with MMPL, the mRNA levels of hTERT and hTR were also inhibited in a dose-dependent manner (Fig. 3D). Collectively, MMPL can directly inhibit the enzymatic activity of telomerase and indirectly diminish its activity through the downregulation of hTERT and hTR expression, suggesting telomerase is a potential target of MMPL in hepatoma cells.

Inhibitory effects of MMPL on uPAR, STAT3, and ERK signaling pathways. Since increased expression of uPAR has been reported in several types of cancer, uPAR is an attractive
therapeutic target for the treatment of cancer metastasis (41). uPAR regulates ECM proteolysis by binding to uPA and promotes cell migration, invasion, and metastasis at cellular surfaces (42). Since MMPL significantly blocked cell motility and the activity of MMPs, reporter assays using a luciferase activity was measured using a microplate reader after reacting the lysates with luciferin as a substrate. SK-Hep1 cells were treated with MMPL (0, 20 and 40 µg/ml) in media containing 1% FBS for 24 h. Total cell lysates were harvested and separated by SDS-PAGE as described in Materials and methods. The expression levels of uPAR, p-STAT3 (Tyr705), STAT3, p-ERK (Thr202/Tyr204), ERK, p-Akt (Ser473), Akt, p-JNK (Thr183/Tyr185), JNK, p-p38 (Thr180/Tyr182), p38, and IκB were detected by specific antibodies. α-tubulin was used as a loading control. (C) The graph illustrates the quantitative analysis data of immunoblot band intensities shown in (B). Data shown are normalized to α-tubulin expression and expressed as the means ± SEM (n=3). *p<0.05, **p<0.01, and ***p<0.001 relative to the MMPL-untreated control group.

Taken together, these results suggest that MMPL can attenuate transactivation of uPAR in hepatoma cells.

Many signaling pathways, such as uPA/uPAR, Janus kinase (JAK)/STAT, phosphatidylinositol-3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and nuclear factor kappa-B (NF-κB), are involved in human cancer progression (43-46). To examine how MMPL suppresses the metastatic ability of hepatoma cells by targeting signaling pathways, the expression levels of phosphorylated STAT3, MAPKs, Akt, and inhibitor of κBα (IκBα) were measured using immunoblotting analysis after incubating SK-Hep1 cells with indicated concentrations of MMPL (0, 20 and 40 µg/ml) for 24 h. As shown in Fig. 4B and C, phosphorylation levels of STAT3 at Tyr705 were dose-dependently suppressed by MMPL in SK-Hep1 cells without changing the total protein levels of STAT3. In addition, among three conventional MAPKs (ERK, JNK, and

Figure 4. Effects of MMPL on uPAR, ERK, and STAT3 signaling pathway. (A) Following transfection with pcDNA3.1-uPAR (-682/+27)-luc and gWIZ-GFP as an internal control, SK-Hep1 and Huh7 cells were treated with MMPL (0, 20, 40, 60 and 80 µg/ml) in media containing 10% FBS for 24 h. Total cell lysates were prepared using passive lysis buffer and uPAR luciferase activity was measured using a microplate reader after reacting the lysates with luciferin as a substrate. (B) SK-Hep1 cells were treated with MMPL (0, 20 and 40 µg/ml) in media containing 1% FBS for 24 h. Total cell lysates were harvested and separated by SDS-PAGE as described in Materials and methods. The expression levels of uPAR, p-STAT3 (Tyr705), STAT3, p-ERK (Thr202/Tyr204), ERK, p-Akt (Ser473), Akt, p-JNK (Thr183/Tyr185), JNK, p-p38 (Thr180/Tyr182), p38, and IκB were detected by specific antibodies. α-tubulin was used as a loading control. (C) The graph illustrates the quantitative analysis data of immunoblot band intensities shown in (B). Data shown are normalized to α-tubulin expression and expressed as the means ± SEM (n=3). *p<0.05, **p<0.01, and ***p<0.001 relative to the MMPL-untreated control group.
and activates other proteases, such as MMPs, leading to plasminogen to plasmin; plasmin then degrades the ECM progression and metastasis (62). uPA, a serine protease, binds aspartyl-, and cysteine proteases, play critical roles in cancer cells (23,61).

The potential to treat liver cancer without affecting normal tissues and organs often limits their use. Since side effects may therefore be a good candidate to improve HCC treatment. In previous studies, normal tissues and organs have been used as a model for cancer treatment (52). Natural products such as curcumin, berberine, and baicalein are rich reservoirs due to their structural and chemical diversity; therefore, the discovery of drug candidates by screening natural products is considered to be an effective strategy for the development of new anticancer drugs (53,54). Natural products-derived chemotherapeutic drugs have shown cancer chemopreventive activity whereas many of common chemotherapy drugs such as doxorubicin, 5-FU, bleomycin, and cyclophosphamide cause toxic side-effects as a results of chemotherapeutic treatments (55-59). Therefore, the discovery of drug candidates by screening natural products is considered to be an effective strategy for the development of new anticancer drugs. This study found that MMPL inhibits cell migration and invasion by inhibiting MMPs and uPAR via the regulation of the ERK and STAT3 pathways; MMPL may therefore be a good candidate to improve HCC treatment.

In addition, the toxicity of many anticancer agents on normal tissues and organs often limits their use. Since side effects occur with almost all anticancer agents (60), novel anticancer agents must overcome this obstacle. In previous studies, normal monkey kidney Vero cells were not susceptible to the cytotoxicity of the methanol extract of *Myrmecodia platytyrea* Becc. with an IC$_{50}$ value of 0.76 mg/ml, and oral administration of the extract was not harmful to mice. However, the extract did not affect the levels of IkB. Taken together, these results suggest that the anticancer effects of MMPL in hepatoma cells are regulated through inhibition of the ERK and STAT3 pathways.

Discussion

HCC, a primary malignancy of the liver, is now the third leading cause of cancer death worldwide (50). There has been increasing interest in characterizing the molecular mechanisms of HCC in order to develop novel therapeutic approaches (51). In this study, the suppressive action of MMPL as a potential anticancer agent was explored in hepatoma cells.

Recently, the development and design of drugs affecting multiple targets or pathways have emerged for cancer treatment (52). Natural products such as curcumin, berberine, and baicalein are rich reservoirs due to their structural and chemical diversity; therefore, the discovery of drug candidates by screening natural products is considered to be an effective strategy for the development of new anticancer drugs. This study found that MMPL inhibits cell migration and invasion by inhibiting MMPs and uPAR via the regulation of the ERK and STAT3 pathways; MMPL may therefore be a good candidate to improve HCC treatment.

In addition, the toxicity of many anticancer agents on normal tissues and organs often limits their use. Since side effects occur with almost all anticancer agents (60), novel anticancer agents must overcome this obstacle. In previous studies, normal monkey kidney Vero cells were not susceptible to the cytotoxicity of the methanol extract of *Myrmecodia platytyrea* Becc. with an IC$_{50}$ value of 0.76 mg/ml, and oral administration of the extract was not harmful to mice. However, the extract was toxic to human hepatoma HepG2 cells with an IC$_{50}$ value of 0.07 mg/ml, suggesting *Myrmecodia platytyrea* Becc. has the potential to treat liver cancer without affecting normal cells (23,61).

Proteolytic enzymes, including metallo-, serine-, aspartyl-, and cysteine proteases, play critical roles in cancer progression and metastasis (62). uPA, a serine protease, binds to uPAR with high affinity and facilitates the conversion of plasminogen to plasmin; plasmin then degrades the ECM and activates other proteases, such as MMPs, leading to tissue remodeling for cancer invasion and progression (62). It has been shown that high expression levels of MMPs and uPA correlate with aggressive behavior of HCC growth and metastasis (35,63). Therefore, suppressing the expression or activity of these proteases has been considered a valuable target against HCC. In this study, MMPL clearly inhibited the migration and invasion of SK-Hep1 cells (Fig. 2). Furthermore, MMPL suppressed protein expression levels of uPAR as well as the enzymatic activity of MMP-2 and MMP-9 in a dose-dependent manner (Figs. 3 and 4). This indicated that MMPL inhibits the metastatic activity of hepatoma cells through the downregulation of uPAR expression, thereby contributing to the decrease in MMP activity. Further studies are needed to confirm whether the reduction of MMP-2 and MMP-9 activity is due to their decreased protein expression.

Cancer metastasis is regulated by complex molecular mechanisms. Recent studies have demonstrated that PI3K/Akt and MAPKs, such as ERK, JNK, and p38, are involved in regulating the expression of MMPs, uPA, as well as uPAR. Furthermore, the STAT3 signaling pathway plays a critical role in the regulation of MMPs and uPAR. These studies imply that the inhibition of these pathways represents a potential target for new anti-metastasis therapies. In the present study, MMPL inhibited the phosphorylation levels of ERK at Thr202/Tyr204 and STAT3 at Tyr705 in a dose-dependent manner, but it did not inhibit phosphorylation of JNK at Thr183/Tyr185 or p38 at Thr180/Tyr182 (Fig. 4B). Collectively, these results suggest that the anti-metastatic activity of MMPL is mediated through the inhibition of the ERK and STAT3 pathways.

In most normal cells, telomerase activity is minimal after embryonic differentiation except in germ cells, stimulated lymphocytes, and stem cells (8,64,65). Therefore, the length of telomeres decreases with age and normal cells have a limited capacity to divide. However, upregulated telomerase activity has been detected in most cancer cells and contributes to the proliferation of cancer (9). In addition, cancer initiating cells are likely to have short telomeres compared with normal cells, suggesting cancer cells are more susceptible to telomerase inhibition (66). Therefore, telomerase is considered to be a useful target in cancer treatment. Two major components of telomerase, hTERT and hTR, remain suitable targets since their expression and activity are essential for the proper functioning of telomerase (67,68).

Many signaling pathways such as PI3K/Akt/mTOR, MAPK, and JAK/STAT are involved in the regulation of telomerase expression and activity (69-71). It has been reported that ERK1/2 promotes the expression and activation of TERT by stimulating Ets-1, Sp-1, and c-Myc transcriptional factors (69,72). Furthermore, STAT3 activation induces TERT expression by binding to the TERT gene promoter region in various cancer cell lines (71). In the present study, MMPL suppressed telomerase activity and mRNA levels of hTERT and hTERT (Fig. 5). In addition, the phosphorylation levels of ERK and STAT3 were inhibited by MMPL (Fig. 4B and C). Taken together, these data suggest that MMPL inhibits telomerase expression and activity by suppressing the ERK and STAT3 pathways. Moreover, MMPL can directly inhibit telomerase activity (Fig. 3C). MMPL might represent a promising new candidate for anti-telomerase therapies.
Phytochemical investigation of *Myrmecodia platytyrea* Becc. has been poorly studied. The plant has been reported to possess several compounds such as liquiritigenin, stigmasterol, morindolide, calycosin, 2-(2-methylbutyryl) phloroglucinol glucoside, and an acylated flavanone (22). Liquiritigenin is known to downregulate MMP-2/9 and PI3K/AKT signaling pathway that are involved in cancer metastasis (73). Calycosin also inhibits breast cancer cell growth by regulating p38 and AKT signaling pathways (74). In addition, stigmasterol induces apoptosis in human hepatoma cells (75). Since MMPL exhibits anticancer activity by inhibiting ERK and STAT3 signaling pathways rather than regulating p38 and AKT activities, further studies are needed to identify the possible active ingredient of the plant for anticancer effects.

Taken together, the results of this study suggest that MMPL has anticancer and anti-metastatic properties in HCC, therefore, MMPL can serve as an effective therapeutic agent for treating human liver cancer.

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